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(54) Title: A METHOD AND A TOBRAMYCIN AEROSOL FORMULATION FOR TREATMENT, PREVENTION AND CONTAINMENT OF TUBERCULOSIS

(57) Abstract

A method for treatment, prevention and containment of acute and chronic tuberculosis using a preservative-free concentrated tobramycin aerosol formulation delivering tobramycin to the lung endobronchial space including alveoli in an aerosol having mass medium average diameter predominantly between 1 to 5 μ. The method comprises administration of tobramycin in concentration one to ten thousand times higher than the minimal inhibitory concentration of *Mycobacterium tuberculosis*. A method for containment of and decreasing infectivity periods of tuberculosis patients to shorter periods of time.

A METHOD AND A TOBRAMYCIN AEROSOL FORMULATION FOR TREATMENT, PREVENTION AND CONTAINMENT OF TUBERCULOSIS

BACKGROUND OF THE INVENTION

Field of Invention

The current invention concerns a method for treatment, prevention and containment of tuberculosis using a tobramycin aerosol formulation. In particular, the invention concerns a preservative-free formulation containing concentrated tobramycin dissolved in full strength or diluted saline adjusted to pH between 5.5 and 7.0. The tobramycin aerosol formulation delivers tobramycin to the lung endobronchial space of airways including alveoli in an aerosol having mass medium average diameter predominantly between 1 to 5 μ. The method for treatment and prophylaxis of acute and chronic tuberculosis caused by the *Mycobacterium tuberculosis* comprises administration of tobramycin in concentration one to ten thousand times higher than the minimal inhibitory concentration of *Mycobacterium tuberculosis*. The invention additionally provides a method for containment of infectivity of tuberculosis patients to shorter periods of time.

BACKGROUND ART AND RELATED ART DISCLOSURES

Mycobacterium tuberculosis or *Tuberculum bacilli* (T. B.) grows in the endobronchial space and is found in the sputum of infected individuals. During exacerbations of infection, such growth also occurs in the alveoli.

Tuberculosis is a highly infectious disease that is characterized by the inflammation and progressive destruction of lung tissue. The debilitation of the lungs in patients with tuberculosis is associated with accumulation of purulent sputum produced as a result of chronic endobronchial infections caused by *Mycobacterium tuberculosis*. Nearly all individuals suffering from tuberculosis eventually die of respiratory failure.

In order to be therapeutically effective, the majority of aerosolized drug particles should not have larger mass medium average diameter (MMAD) than between 1 and 5 μ. When the aerosol contains a large number of particles with a MMAD larger than 5 μ, these are deposited in the upper airways decreasing the amount of the drug delivered to the site of infection in the lower respiratory tract.

In view of the above described disadvantages of systemic treatment of tuberculosis, it would be highly advantageous to provide a method for treatment and prevention of *Mycobacterium tuberculosis* infections by a locally administered anti-tuberculosis drug in an aerosol form.

It is, therefore, a primary objective of this invention to provide a method and a regimen for treatment, prevention and containment of infectivity of tuberculosis by a concentrated aerosol formulation containing a sufficient amount of an anti-*Mycobacterium tuberculosis* drug to eradicate or substantially suppress *Mycobacterium tuberculosis* bacterium.

SUMMARY

One aspect of the current invention is a method for treatment, prevention and containment of pulmonary tuberculosis or other infections caused by *Mycobacterium tuberculosis, Mycobacterium bovis* or other mycobacteria by administering to a subject requiring such treatment a formulation comprising about a one to ten thousands times higher concentration of tobramycin than is its minimal inhibitory concentration.

Another aspect of the current invention is a method for treatment, prevention and containment of pulmonary tuberculosis caused by *Mycobacterium tuberculosis,* by administering to a subject requiring such treatment twice daily, for at least five days, a formulation comprising about 8 to about 80 mg/mL of tobramycin dissolved in a full or quarter normal saline having a pH between 5.5 and 7.0 and delivered by a jet or ultrasonic nebulizer in 5 mL

Figure 2 illustrates a structure of tobramycin.

Figure 3 depicts a jet nebulizer suitable for aerosolization of the concentrated tobramycin solution.

Figure 4 is a graph depicting results of killing curve assay for tobramycin compared to isoniazid.

DETAILED DESCRIPTION OF THE INVENTION

The current invention is based on finding that tobramycin in concentrations between 8 and 16 μg/mL is able to substantially and continuously reduce a count of *Mycobacterium tuberculosis* responsible for highly infectious pulmonary tuberculosis. In the killing curve assay following the treatment with tobramycin, the count of *Mycobacterium tuberculosis* expressed in colony forming units (CFU), decreased from 10^6 to about 10^2.

The current invention, therefore, concerns a method for prevention and treatment of pulmonary tuberculosis and for containment and reduction of an infected individual's infectivity. The invention also concerns a concentrated tobramycin formulation suitable for efficacious delivery of tobramycin by aerosolization into endobronchial space. The method of the invention is most preferably suitable for prevention of development of pulmonary tuberculosis in individuals subjected to contact with a tuberculosis infected subject, for treatment of the subject already infected with tuberculosis as well as for containment and decreasing the infectivity time of the infected individuals.

The method involves providing to the individual in need of any of the above mentioned treatment an aerosolized tobramycin in concentration sufficient to decrease the count of *Mycobacterium tuberculosis* to levels lower than 10^3 and to contain and decrease the infectivity of tuberculous patients to several days rather than weeks or months.

High concentrations of tobramycin administered to the lungs by aerosolization result in maximization of sputum levels of tobramycin and in minimization of tobramycin serum levels. Thus, administration of tobramycin by

for general *M. tuberculosis* infections.

One aspect of the invention is a therapy of the highly infectious pulmonary tuberculosis which devastates the patient and without treatment is often fatal. While some effective drugs are available and efficacious in treatment of tuberculosis, they often lead to the development of the *M. tuberculosis* bacterium resistance and with time become either much less or completely ineffective. Moreover, as described above, the systemic treatment of tuberculosis leads to development of various undesirable side effects.

The new aerosol therapy of the invention enables localized delivery of the effective anti-tuberculosis drug tobramycin to a site where the bacterium resides and in this way enables the suppression and eradication of the tuberculosis.

In another aspect, the invention is useful for prophylactic purposes. Due to its high infectivity, tuberculosis is highly contagious even under most normal circumstances, that is by being subjected to a contact with the infected individual. Since the infection is spread by small droplets generated by coughing of the infected individual, there is no practical way of avoiding it. The method for prophylaxis, according to the invention, treat the individual being in contact or being previously subjected to the contact with a tuberculosis patient with sufficiently high concentration of tobramycin able to prevent growth of *M. tuberculosis* bacterium.

One other important aspect of the invention is the method for containment and decreasing infectivity of tuberculosis patients.

Tuberculosis is a very infectious disease, spread and transmitted by coughing which creates small aerosol droplets containing *Mycobacterium tuberculosis* bacteria. These bacteria come from any part of the airways in upper lung or in lower lung or alveoli of the lung. In addition, the larynx of tuberculosis patients is highly infectious and the patients, therefore, need to be hospitalized in an

its minimum inhibitory amount. Tobramycin is nebulized predominantly into particle sizes which are delivered to the terminal and respiratory bronchioles and alveoli (lower lungs) where the *M. tuberculosis* is present in patients infected with tuberculosis as well as in the upper lung and larynx where *M. tuberculosis* is also present and which is responsible for patient's infectivity. Moreover, high concentration of aerosolized tobramycin comes into contact with the sputum, penetrates it and kills bacteria present there.

Subdivision and structure of intrapulmonary airways (lower lung) are seen in Figure 1. *M. tuberculosis* is present in the upper airways, in bronchi and bronchioli, particularly in terminal and respiratory bronchioles. During exacerbation of infection, bacteria can also be found to be present in alveoli. Any therapeutic formulation must be, therefore, delivered to the terminal bronchioles and to alveoli.

I. Method for Treatment and Prophylaxis of Tuberculosis

The current invention provides a safe and efficacious method for treatment and prophylaxis of pulmonary *Mycobacterium tuberculosis* infections.

Primary requirements for any method of treatment and prophylaxis as well as for any aerosolized formulation are its safety and therapeutic and prophylactic efficacy. Lower cost, practicality of use, long shelf-life of the formulation, its storage and manipulation of the nebulizer are also definite consideration for any treatment. The current method and the aerosol formulation used in the method provides all the above advantages.

Briefly, the method for treatment for prophylaxis comprises administering to a patient diagnosed with a pulmonary tuberculosis or a subject being in contact with the patient having *Mycobacterium tuberculosis* present in the sputum, about 40 to about 800, preferably about 300 mg of nebulized tobramycin once or twice daily for at least

easily transmitted by coughing which creates small aerosol droplets containing T.B. bacteria. These bacteria can come from any part of the airways or alveoli of the lung. Most often, however, they are from the highly infectious larynx. The method for suppressing infectivity utilizes locally administered high concentrations of tobramycin to decrease both the infectivity as well as the period of time during which the patient is infective even after initiation of therapy. The method essentially utilizes local administration, by aerosol, by spray or by smearing of the patient's mouth and throat with the solution of concentrated tobramycin comprising between 100 and 600 mg of tobramycin as many times daily as necessary.

Tobramycin is an antibiotic having a chemical structure seen in Figure 2. Tobramycin has now been found to possess anti-*Mycobacterium tuberculosis* bactericidal activity responsible for an observable decrease in quantitative spectrum counts of *Mycobacterium tuberculosis* during the first two days of therapy according to the invention. The early bactericidal activity of tobramycin is its inherent property which is comparable to tobramycin activity measured *in vitro*, in logarithmic plot cultures. Results of the *in vitro* study performed according to Am. Rev. Respir. Dis., 21:939 (1980) are seen in Figure 4.

Figure 4 is a graph showing results of a killing curve assay for tobramycin. Tested tuberculosis strain, *M. tuberculosis* H37Rv(lux), contains a luciferase reporter construct, and has similar growth characteristics to *M. tuberculosis* H37Rv. For this study, cultures containing 10^6 CFU/mL of *M. tuberculosis* H37Rv (lux), grown in Middlebrook 7H9 medium were incubated with various concentrations of antibiotic tobramycin or a known anti-tuberculosis drug isoniazid (INH). Bactericidal activity of these two drugs, at the indicated concentrations, were compared to cultures grown without antibiotics, serving as a growth control. Aliquots were withdrawn at the indicated timepoints for quantitation by serial dilution.

in the alveolar and bronchial space, rapid killing of the extracellular population of bacilli is assured. Tobramycin delivered by the aerosol route achieves significant concentrations in lung parenchyma. An aerosol drug delivered directly to the bronchial and alveolar space is therefore a useful addition to current antituberculosis drug regimens.

Mycobacterium tuberculosis encompasses a variety of multidrug-resistant strains which are more or less resistant to treatments with various drugs such as isoniazide, streptomycin, kanamycin, rifampin and ethanbutol. Many of these strain were shown to be sensitive and nonresistant to treatment with tobramycin.

Results of the comparative resistance study are seen in Table 1. In Table 1, drug resistance was determined by BACTEC test commercially available from Becton-Dickinson. BACTEC test which measures growth of bacteria by detecting the utilization of C^{14} labeled substrate, was performed with the following drug concentrations (μg/mL): isoniazid 0.4 μg/mL; rifampin 2 μg/mL; streptomycin 6.0 μg/mL; kanamycin 5.0 μg/mL and ethanbutol (EMB) 7.5 μg/mL. Tobramycin concentrations needed to achieve minimal inhibitory concentration (MIC; μg/mL) are seen in Table 1.

TABLE 1

Activity of Tobramycin Against Drug-Sensitive Strains of *M. tuberculosis*

Drug Sensitive Strain	Tobramycin MIC (μg/mL)
H37Rv	4
TN994	4
TN2351	8
TN3183	4
TN675	4
TN2524	4
TN4259	4
TN1008	4
TN1039	4
TN3979	4

according to the invention. Minimal inhibitory concentration of systemically administered tobramycin is believed to be out of the safe range of tobramycin in serum. The maximum safe concentration range for tobramycin is between 8-10 μg/mL in the serum. However, to achieve the concentration which was found to be bactericidal, that is at least 8 μg/mL and above in the lungs and sputum, tobramycin would have to be present in the systemic circulation in a much higher concentration than the maximum safe concentration of 8-10 μg/mL. Absorption and transport of systemically administered tobramycin into lung parenchymal tissue is very low, in a ratio of probably less than 100:1 systemic/lung concentration. Consequently, the minimal inhibitory concentration which would need to be present in the systemic circulation would have to be at least 800-1000 μg/mL. Such concentrations are considered to be unsafe. For these reasons, tobramycin is generally not used for tuberculosis treatment.

While tobramycin concentrations of 8 μg/mL are difficult to achieve in sputum with parenteral administration, such and much higher concentrations may be achieved in the airways with minimal systemic toxicity using aerosol delivery.

Several studies have documented the safe delivery of high concentrations of tobramycin to the sputum using an aerosol route. Much of this work has been done toward treatment of chronic lung infections with *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients and is described in U.S. patent 5,508,269, hereby incorporated by reference. A multicenter, double-blind, placebo-controlled cross-over trial in 71 CF patients of aerosolized tobramycin 600 mg three times daily (tid) for endobronchial infections due to *P. aeruginosa* demonstrated a significant reduction in sputum density of *P. aeruginosa* as well as improved spirometry in the treatment group.

A 600 mg tid regimen of aerosolized tobramycin was thus found to be both safe and efficacious in CF patients.

untreatable.

II. Tobramycin Aerosol Formulation for Treatment and Prophylaxis of Tuberculosis

Aerosolized tobramycin formulation of the invention is formulated for efficacious delivery of concentrated tobramycin to the lung endobronchial space for treatment and prophylaxis of tuberculosis. The formulation has adjusted salinity to permit generation of tobramycin aerosol well-tolerated by patients. Further, the formulation has balanced osmolarity ionic strength and chloride concentration. The formulation utilizes a smallest possible aerosolizable volume, preferably from about 3 to about 7 mL, most preferably 5 mL, able to deliver effective dose of tobramycin to the site of the infection. The tobramycin aerosolized formulation does not impair negatively the functionality of the airways and does not cause undesirable side effects.

The tobramycin formulation to be used for treatment or prophylaxis of tuberculosis according to the invention, contains from 40-800, preferably 100-400, and most preferably 300 mg of tobramycin sulfate per 5 mL of the quarter normal saline. This corresponds to 8-160, preferably 20-80 and most preferably 60 mg/mL of tobramycin, which represents less than about 10^4 times minimal inhibition concentration of tobramycin. This formulation, delivered in nebulized form, suppresses the tuberculosis infection in endobronchial space rapidly within the several hours to several days.

Typically, the selected amount of tobramycin, as described above, is dissolved in 5 mL solution of saline, preferably a diluted, typically quarter normal saline, containing about 0.225% NaCl. The quarter normal saline has been found to be a most suitable vehicle for delivery of tobramycin into endobronchial space.

The effective dose of tobramycin will depend on its intended use. Tobramycin is optimally used in 40-100, preferably 60 mg/mL dosage for treatment of tuberculosis in

aerosol solution having pH between 4.5 and 5.5 will occasionally cause this problem. The aerosol solution having a pH between 5.5 and 7.0 is considered safe. Any aerosol having pH greater than 7.0 is to be avoided as the body tissues are unable to buffer alkaline aerosols and result in irritation and bronchospasm. For the safety of the patient, it is preferred that pH of the formulation is between 5.5 and 7.0, most preferably between 5.5 and 6.5.

The pH is also important for stability of the formulation. At pH greater than 7.0 degradation of tobramycin occurs. To determine the stability of the tobramycin formulation vis-a-vis its pH, four studies, described in Examples 3 and 4 were performed.

In the first stability study, described in Example 3, accelerated stability testing of 60 mg/mL tobramycin, at 40°C temperature and at pH 7.0, showed after 35 days, yellowing of the solution indicating the presence of chromophore degradation product. Results are seen in Table 5. This finding was unexpected and not predicted by the literature on aminoglycoside degradation (Drug Develop. Industr. Pharm., 18:1423-36 (1992)).

The above observed reaction was less apparent when the same testing was done at pH 5.5 or 6.5 (Table 5). At such pH, the degradation was either not present or was much slower. The optimal pH for the aerosol formulation was, therefore, determined to be between pH 5.5 to pH 6.5.

In two extended stability studies described in Example 4, the formulation was stable for more than 6 months at temperature 5°C at about pH 6. Results are seen in Tables 6 and 7. Less stable was the formulation stored for 6 months at 25°C, where the color formation increased from 15 KS units to 52 KS units. Results are seen in Tables 8 and 9.

The formulation of the invention is nebulized predominantly into particle sizes allowing a delivery of the drug into both upper and lower lung including the terminal and respiratory bronchioles where the

The nebulizer must be able to nebulize the formulation of the invention into aerosol particle size predominantly in the range from 1-5 μ. Predominantly in this application means that at least 70%, but preferably more than 90%, of all generated aerosol particles are within 1-5 μ range.

Two types of nebulizers, jet and ultrasonic, can produce and deliver particles between the 1 and 5 μ particle size. A jet nebulizer works by air pressure to break a liquid solution into aerosol droplets. An ultrasonic nebulizer works by a piezoelectric crystal that shears a liquid into small aerosol droplets.

While the range variety of nebulizers is available, only limited number of these nebulizers are suitable for the purposes of this invention. One representative nebulizer suitable for purposes of this invention is illustrated in Figure 3.

Figure 3 shows a representative jet nebulizer 10 useful for aerosolization of tobramycin aerosol having particle size predominantly in 1-5 μ region. Nebulizer (10) consists of the outside case (14), mouthpiece (30), nebulizer cup (22) covered with cap 16, Venturi chamber (28), air supply tube (24), liquid medicine cup (22) and baffle (18).

The liquid formulation is placed into the nebulizer cup (22) by removing and replacing the cup's cap (16). The cap (16) has one or more air intake holes (20) that allow entrainment of room air to Venturi chamber (28). Venturi chamber (28) allows entrained room air to mix with aerosol to increase drug delivery. Air supply tube (24), typically (8 l/M) is connected to nebulizer's liquid medicine cup (22). Air goes through the cup (22) into jet nebulizer orifice (26) where it creates aerosol by shearing the liquid solution into small threads of liquid that shatter into small particles when it hits a baffle (18). The nebulizer 10 further comprises a mouthpiece (30) for inhalation of aerosol. The mouthpiece contains flapper valve (12) to allow exhalation. The mouthpiece (30) is

tobramycin, measured as a mean peak sputum concentration. In this study, only 13% of all treated patients had sputum concentrations lower than 128 μg/g. All other patients (87%) achieved sputum concentrations larger or equal to 128 μg/g.

The second study, described below, was designed to determine *in vitro* which nebulizers meet criteria that are important for delivery of aerosolized antibiotics. Both ultrasonic and jet nebulizers were studied.

The third study was designed to determine the pharmacodynamics of tobramycin in the sputum which is a measure of the efficacy of the aerosol delivery.

An *in vitro* comparative study evaluated a variety of commercially available jet nebulizers, including among others, the Acorn II® by Marquest, T-Updraft® by Hudson, Sidestream® by Medicaid, and Pari LC® by Pari. The PulmoAide® compressor was chosen because of its reliability and widespread use.

Studies of these nebulizers revealed that most of them are relatively inefficient in delivering an inhalable mist. The three chosen nebulizers used in the clinical protocols, the ultrasonic DeVilbiss 99, the Pari LC jet and the Medicaid Sidestream jet, have shown properties suggesting that they could possibly deliver tobramycin aerosol into endobronchial space. Of the three, two jet nebulizers were clearly superior to the ultrasonic DeVilbiss nebulizer. Therefore, they have been evaluated to determine which one of them could provide the greatest amount of drug to the airways and two jet nebulizers were found to meet the requirements.

A comparative characteristics of the Ultraneb 99 DeVilbiss (ultrasonic) and two of the jet nebulizers, the Sidestream and the Pari LC with the PulmoAide compressor, showing the best characteristics, are listed in Table 3.

as much drug, that is 600 mg of tobramycin compared with 300 mg for the two jet nebulizers, for delivery of 33 mg/mL.

The major limitation of the Ultraneb 99 (DeVilbiss) ultrasonic nebulizer used for delivery of tobramycin formulation are its high-cost, waste of the drug and inconvenience. As seen from Table 3, this nebulizer requires 30 mL of the drug solution, and it has large, 1200 mL aerosol reservoir. In order for tobramycin aerosol therapy to be widely available and used by patients with tuberculosis in ambulatory or home setting, a more efficient and easier to use nebulizer is needed.

Although all suitable nebulizers are intended to be within the scope of this invention, a jet or ultrasonic nebulizers, preferably modified jet nebulizer Pari LC Plus, equipped with Pulmo-Aid compressor are most preferred for the method of the invention.

IV. Efficacy

The efficacy of the method of the invention for therapy, prophylaxis and containment of infectivity depends on the delivery of the aerosolized tobramycin formulation and is determined and predicted by the presence and concentration of tobramycin in sputum. If the concentration of tobramycin found in sputum is sufficient to suppress the *Mycobacterium tuberculosis* infection, then the combination of the regimen/formulation/nebulizer is efficient for treatment, prophylaxis or containment of the infection. The most efficient method is when the regimen of administration regimen delivers the amount of aerosolized drug which is therapeutically effective in the great majority (>90%) of patients, where all or almost all of the drug is delivered into the site of infection, and where delivered drug amount is sufficient to suppress the *Mycobacterium tuberculosis* in sputum.

The measure of the efficacy in this instance is the finding sufficient amount of the nebulized drug in the sputum in >90% of the patients in the treated population.

Hot water for injection was thoroughly flushed (WFI) through 20 L Millipore product vessel. Tobramycin potency (g/L) was assayed and tobramycin was added to product vessel. The amount of tobramycin was weighed accurately into a wide mouth specimen bottle and labeled. 11.25 kg of WFI was dispersed into a clean 20L Millipore product vessel. With moderate agitation, 33.75 g sodium chloride, USP, was slowly added and mixed until dissolved. WFI was added to the product vessel to 12 Kg and mixed for 5 minutes, under continual mixing, 100 mL 5 N H_2SO_4 (sulfuric acid) was carefully added for each liter of WFI in the final formulation. Product vessel was sparged with nitrogen (N_2). After approximately 15 minutes of sparging, dissolved oxygen (O_2) was measured by continuous monitoring of dissolved oxygen in the tank, using an oxygen probe. The amount of the dissolved O_2 was continued to be monitored until five (5) consecutive measurements reached levels of $\leq$ 3 ppm of dissolved O_2. With continuous sparging of N_2 and under moderate mixing, the tobramycin was added and mixed until dissolved. Twenty mL sample of formulation was removed and pH was measured. The formulation was adjusted to a final pH 6.0. An aliquot of product formula was sampled and analyzed for tobramycin concentration, for pH, and for dissolved O_2 (in triplicate). When the batch met quality control testing criteria, the formulation was released and used in clinical setting.

EXAMPLE 2

Tobramycin Formulation - Effect of A Normal and Dilute Saline

This example describes testing performed to determine the effect of normal and quarter strength diluted saline on the aerosolization of tobramycin and on the amount of drug delivered over a ten-minute period.

To test output from a hand-held portable ultrasonic nebulizer, an UltraAirs by Omron was used. This ultrasonic nebulizer has a reusable medication cup that is placed over the ultrasonic crystal. The medication cup was weighed, a

200 would typically be a strong amber color. The human eye can first detect a tint at about a KS scale of 20. Typically a change of KS scale from the 0-20 range (a colorless solution) to a value greater than 200 would be a limiting factor in stability studies even though the drug in the formulation may still be potent.

Since Drug Develop Industr. Pharm., 18:1423-36 (1992) details that the major degradation process for tobramycin is oxygen dependent, the packaging was done in a nitrogen enriched environment. Results are summarized in Table 5.

TABLE 5

Results of 35 Day Stability Study at 40°C

Target pH	N	Time (days)	Actual pH (Mean)	Color KS units (Mean)
5.5	3	0	5.55	13
5.5	5	35	5.51	104
6.5	3	0	6.57	12
6.5	5	35	6.56	107
7.0	3	0	7.07	13
7.0	5	35	7.04	171*

*p< .05 compared to 5.5 and 6.5.

KS units express color changes as described above.

Polyethylene LDPE vials were used for three pH values. Each vial contains 60 mg/mL tobramycin in 5 mL of .225% NS, 5 mL total volume and was stored in foil overpouch nitrogen enriched environment. Color and actual pH testing was done at a time and after a storage for 35 days at 40°C.

The results seen in Table 5 are surprising as a color of the formulation appears to be dependent upon pH of the formulation. The development of the color is an early marker of tobramycin degradation and an undesirable product characteristic. The formulation dependence on the pH shows that the optimal pH for the tobramycin formulation is in the pH 5.5 to 6.5 range.

Furthermore, the rapid coloring of the solution at 40°C teaches that storage at lower temperatures such as 5°C to

Container: Rexene 6010 LDPE
Container Volume: 5 mL
Fill Volume: 5 mL
Storage Condition: 5°C
Sterilization: Aseptic Fill
Overpouch: Preformed Laminated Foil

TABLE 7

Extended Stability Study II

Baseline, 3-Month and 6-Month Data at 5°C

Assay Description	Units	Limits	Initial	3 Mo	6 Mo
pH		5.5-6.5	5.8	6.1	6.0
Tobramycin	mg/mL	54.0-72.0	60.3	59.5	59.4
Chloride as Sodium Chloride	mg/mL	2.02-2.48	2.29	2.30	2.31
Color	KS	0-200	22	20	25
Visual Inspection		Pass	Pass	Pass	Pass
Impurities-Peak D	%	0.00-5.00	ND	ND	0.00
Impurities - Peak X	%	0.00-5.00	ND	4.87	0.00
Annular Oxygen Conc.	%	0.00-8.00	4.87	4.66	4.06

ND = None Detected
Product: 60 mg/mL Tobramycin formulated for inhalation
Container: Rexene 6010 LDPE
Container Volume: 5 mL
Fill Volume: 5 mL
Storage Condition: 5°C
Sterilization: Aseptic Fill
Overpouch: Preformed Laminated Foil

TABLE 9

Extended Stability Study IV

Baseline, 3-Month and 6-Month Data at 25°C

Assay Description	Units	Limits	Initial	3 Mo	6 Mo
pH		5.5-6.5	5.8	6.1	5.9
Tobramycin	mg/mL	54.0-72.0	60.3	60.1	59.4
Chloride as Sodium Chloride	mg/mL	2.02-2.48	2.29	2.30	2.31
Color	KS	0-200	22	52	73
Visual Inspection		Pass	Pass	Pass	Pass
Impurities-Peak D	%	0.00-5.00	0.00	0.03	0.05
Impurities - Peak X	%	0.00-5.00	ND	0.00	0.07
Annular Oxygen Conc.	%	0.00-8.00	4.87	4.38	4.69

ND = None Detected

Product: 60 mg/mL Tobramycin formulated for inhalation

Container: Rexene 6010 LDPE

Container Volume: 5 mL

Fill Volume: 5 mL

Storage Condition: Room Temperature (25°)

Sterilization: Aseptic Fill

Overpouch: Preformed Laminated Foil

As seen from Tables 5-9, color of the tobramycin solution did not change substantial even during extended storage at 5° or 25°C confirming the good stability of the tobramycin solution under the stated study conditions.

After six months at 5°C, neither of two batches had shown any changes outside of set limits. At 25°C storage, the only observed change was the formation of low levels of color, with the two batches showing increases from 15 KS units to 52 KS units and from 22 KS units to 73 KS units.

The formulation at high concentration at the optimum pH of 6 is thus completely stable at 5°C. After six months at 25°C, the formulation is effectively stable, remaining well within an acceptable range of color.

* Timed from completion of aerosol treatment

† < 20 μg/gm

TABLE 11

Serum Concentrations Following Aerosol Administration of 300 mg Tobramycin in 5 mL of 1/4 NS

Subject #	Jet Nebulizer	Serum Tobramycin μg/mL			± % change FEV_1* 30 min*
		Baseline	1 hour*	2 hours*	
001	Sidestream	not detected	0.3	0.2	-2.2%
	Pari LC	not detected	0.2	0.3	-1.2%
002	Sidestream	not detected	0.2	0.2	0.0%
	Pari LC	not detected	0.5	0.4	1.2%
003	Sidestream	not detected	0.9	0.8	-7.6%
	Pari LC	not detected	0.6	0.5	-0.6%
004	Sidestream	not detected	1.2	1.0	-1%
	Pari LC	not detected	0.5	0.4	-2%
005	Sidestream	not detected	1.6	1.0	0.0%
	Pari LC	not detected	0.8	0.7	-5%

* Timed from completion of aerosol treatment

< 20 μg/gm

The results show that an aerosolized tobramycin dose of 300 mg in 5 mL of 60 mg/mL in 1/4 NS achieved the target concentration of > 128 μg/gm in sputum. This dosage was also considered extremely safe because serum concentrations were smaller than < 1.6 μg/mL and were, therefore, well below the recommended peak therapeutic serum concentrations 8-10 μg/mL.

EXAMPLE 6

Safety and Efficacy of Tobramycin Treatment

Selection of Patients

This example describes a study for safety and efficacy of tobramycin formulation for treatment of tuberculosis.

In this study, the efficacy and safety parameters are assessed for tobramycin formulation containing 300 μg of tobramycin administered twice daily for five days to patients with newly diagnosed pulmonary tuberculosis.

The second objective is to determine whether the tobramycin concentration required to achieve a peak sputum concentration of 128 μg/gm or greater is safe and well tolerated by the patient. Safety is defined as a lack of acute bronchospasm and minimal systemic absorption.

All patients with underlying disease of tuberculosis, confirmed at entry by the inclusion/exclusion criteria specified in this protocol, are eligible for enrollment into the study. Investigators at the participating TBC centers select patients that meet all of the inclusion criteria and one of the exclusion criteria.

Eligible patients are admitted to the study center on the day of the study and receive aerosol therapy if they fulfill entrance criteria.

Physical exam is administered by a physician or RC nurse prior to initial aerosol treatment only.

Vital signs, height, weight, oximetry, assessment of current respiratory status and brief medical history are recorded.

Sputum and serum samples are collected to measure baseline tobramycin concentrations.

Patients sat upright and used nose clips during the aerosol administration. The total duration of time and the number of inhalations required to complete the aerosol treatment are recorded. Any evidence of wheezing or respiratory distress are recorded as well as number of rest periods required by the subject because of dyspnea or excessive coughing during the administration period.

Immediately after completing the aerosol therapy, the subject is asked to rinse with 30 mL of normal saline through the mount, gargle for 5-10 seconds and expectorate the rinse. This is repeated for a total of three rinses. Sputum specimens are collected at 10 minutes after rinsing oral cavity and 2 hours after completion of the aerosol drug administration. Serum is collected at 1 and 2 hours after completion of the aerosol drug administration for determination of the tobramycin levels. Spirometry is

WHAT IS CLAIMED IS:

1. A method for treatment, prophylaxis and containment of infectivity of pulmonary tuberculosis by providing a patient in need of such treatment an aerosol formulation comprising concentrated tobramycin, said method comprising a step:

(a) administering to the patient a nebulized aerosol formulation comprising from about 40 to about 800 mg tobramycin once or twice daily for five or more days.

2. The method of claim 1 wherein tobramycin is dissolved in about 3 to about 5 mL of normal or diluted saline adjusted to pH between about 5.5 and 6.5.

3. The method of claim 2 wherein said formulation is administered by a jet nebulizer able to produce aerosol particle sizes predominantly between about 1 and about 5 microns.

4. The method of claim 3 suitable for therapy of pulmonary tuberculosis wherein the treatment comprises administration of tobramycin in concentration from about 100 to about 400 mg twice daily for at least five days or more.

5. The method of claim 4 wherein the treatment comprises administration of tobramycin in concentration of about 300 mg.

6. The method of claim 3 suitable for prophylaxis of pulmonary tuberculosis wherein the treatment comprises administration of tobramycin in concentration from about 80 to about 300 mg twice daily for at least five days or more.

said method achieving an inhibition of at least 95% of susceptible *Mycobacterium tuberculosis* in endobronchial space of lower lungs, upper lung, alveoli, larynx and pharynx of a patient suffering from the tuberculosis.

14. An aerosol formulation suitable for therapy, prophylaxis or containment of *Mycobacterium tuberculosis* infections of a patient suffering from tuberculosis, said formulation comprising from about 40 to about 800 mg of tobramycin dissolved in about 3 to about 7 mL of a normal or diluted saline adjusted to pH between about 5.5 and 7.0 and administered by aerosolization using a jet or ultrasonic nebulizer able to produce aerosol particle sizes predominantly between 1 and 5 μ.

15. The formulation of claim 14 wherein the pH is about 6.0.

16. The formulation of claim 15 wherein the nebulizer is the jet nebulizer.

17. The formulation of claim 16 wherein the nebulizer is the ultrasonic nebulizer.

18. The formulation of claim 16 comprising from about 100 to about 400 mg of tobramycin dissolved in about 5 mL of quarter normal saline solution.

19. The formulation of claim 18 comprising about 300 mg of tobramycin.

FIG. 1

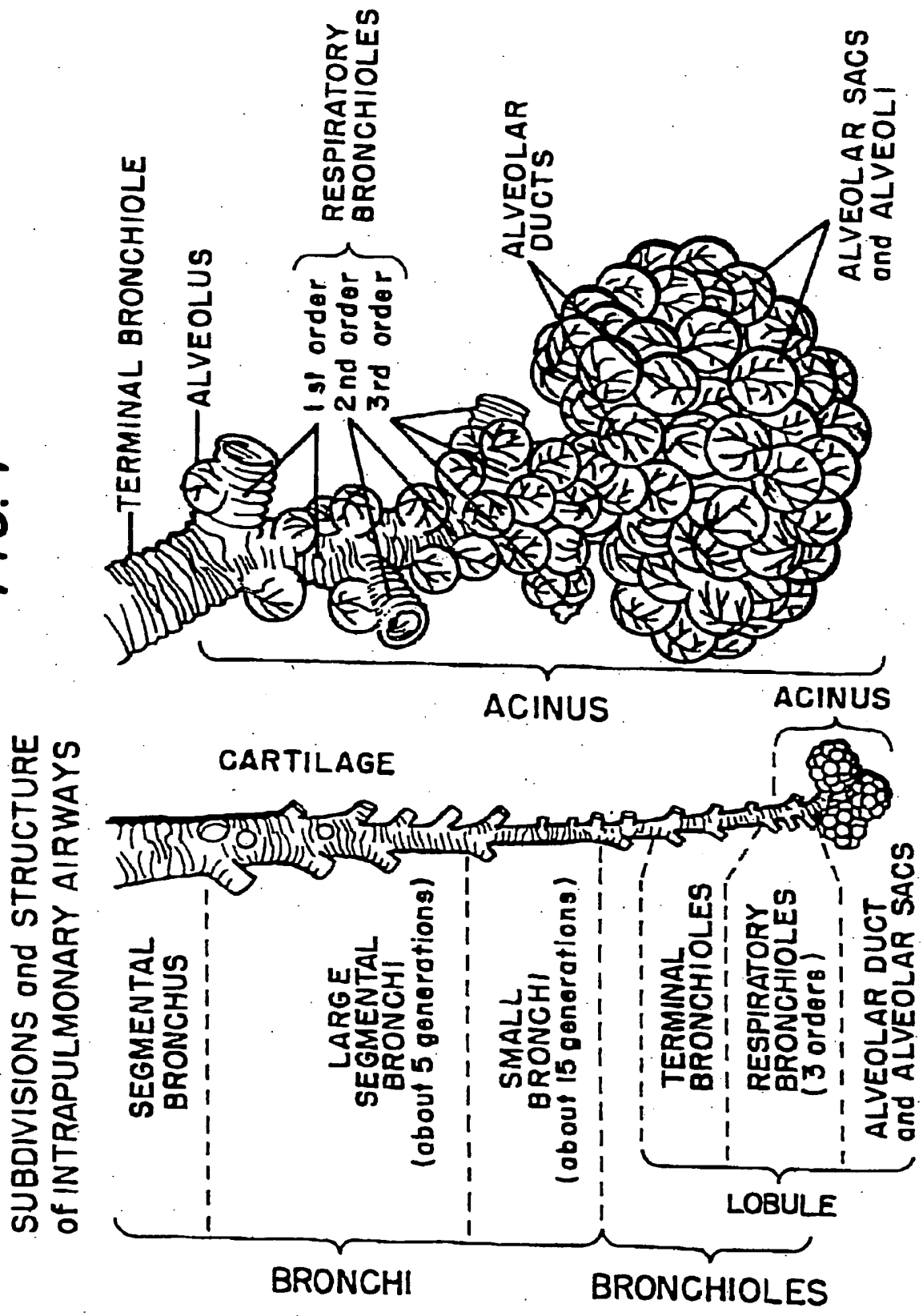
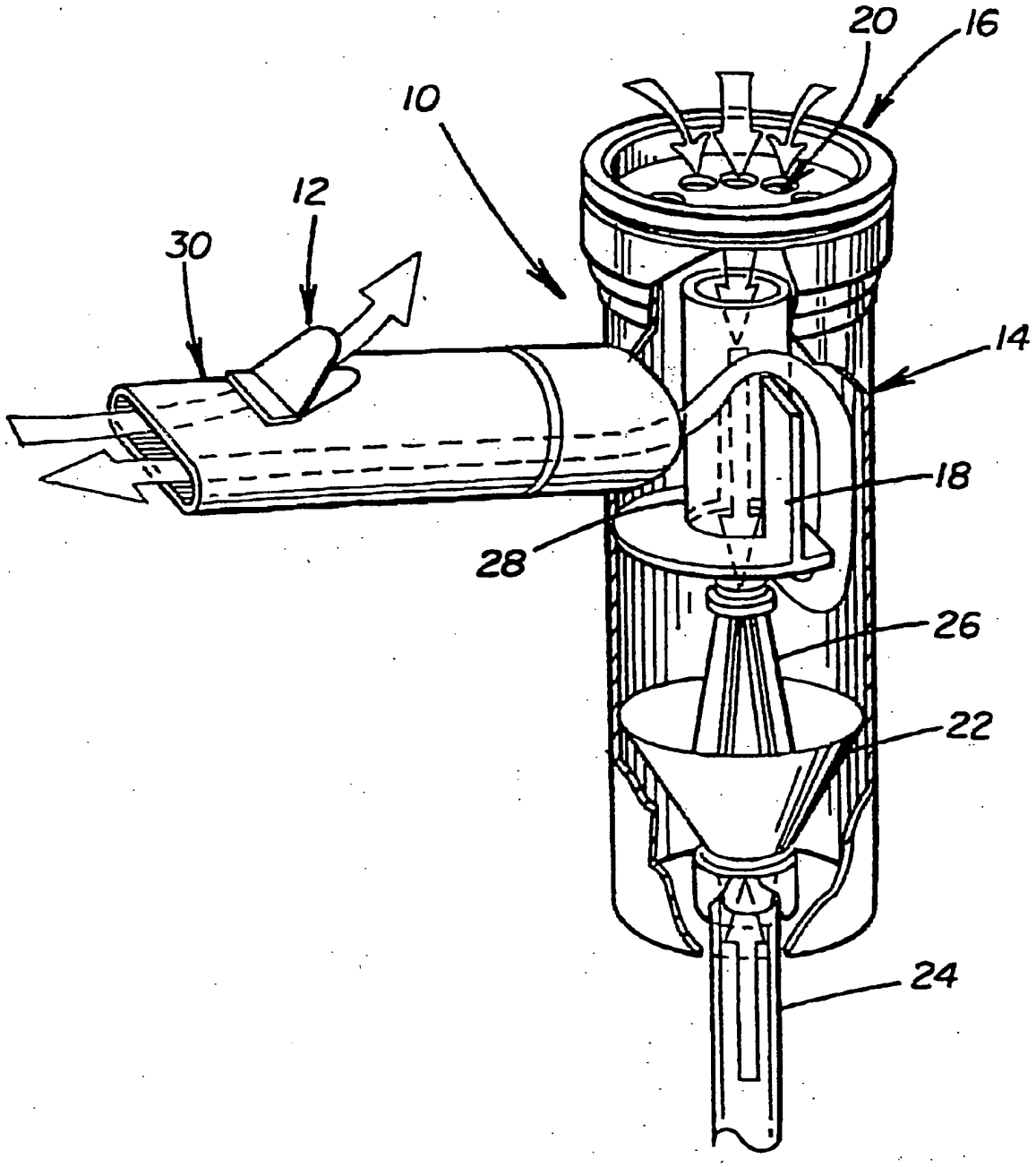


FIG. 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/05179

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/70
US CL :514/35, 36, 38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/35, 36, 38

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,761,402 A (WILLIAMS et al) 02 August 1988, column 15, lines 53-68 and column 16, lines 1-46.	1-13 and 20
Y	US 5,508,269 A (SMITH et al) 16 April 1996, column 13, lines 38-65.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
16 APRIL 1998	23 jun 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ELLI PESELEV Telephone No. (703) 308-1235

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